INBREEDING OF JUNONIA COENIA (LEPID.) THROUGH THIRTY-FIVE SUCCESSIVE GENERATIONS

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Every nature lover marvels over the wonderful coloring and marking of our thousands of different species of butterflies. My interest in these beautiful insects was aroused as a small boy, and now, at three score years, this interest deepens, the longer I am able to carry on experiments.

It is obvious that the many thousands of different patterns evidenced in butterflies were not produced at one time, but are the result of a series of gradual changes. It is a fascinating pursuit to endeavor to determine the factors that are operative in their production, and to produce by experimental means in a short space of time, changes that, in a state of nature are the result of many centuries.

This I have accomplished in a measure, as a result of intensive breeding, with a variety of artificially produced environments.

Experimentation in this field was begun in 1906, in a small sunny room, and with very little time at my disposal. The results of this earlier work were published in various scientific journals. The terrible world war, and consequent business depression, had its effect in inhibiting many scientific activities, and it is only at this time that matters have shaped themselves for a further recording of work carried on since my last published notes.

A brief summary of former experimentation will first be given, but before introducing this I wish to thank Dr. John Comstock, the president of the Southern California Academy of Sciences, for his aid and encouragement in this work. As the result of these experiments is of educational value, I will present all of the original specimens described and illustrated on the two accompanying plates, to one of the local museums, for permanent exhibition.

There seems to have been some misunderstanding by some of my former readers, as to the meaning of the term inbreeding, and an explanation is here in order.

Live-stock specialists follow three distinct methods of breeding, namely, inbreeding, line-breeding and crossing. The terms are self-explanatory.

Inbreeding has been demonstrated to be the best method of rapidly fixing a new point in color or marking.

This is carried on in the following way; a gravid female of the usual type is captured in the open, and imprisoned in a wire cage over a potted plant that is known to be the proper food of the species. This is placed in a sunny spot, and the female proceeds to oviposit. The butterflies finally resulting from these eggs are my first generation. These are mated, (brother with sister, to use the common vernacular.) Successive generations are paired in the same way, care being taken to select examples that show the special points it is desired to preserve.

I have tried line-breeding, by mating two males of the same species, captured in separate localities, with two females captured in a like manner. The progeny of these two matings were kept separate,—one designated line A, the other line B.

Males of line A were mated with females of line B, and vice versa. Each successive generation was mated in the same manner.

This form of breeding was found to be productive of little result, and was much more difficult to carry on. One drawback re-

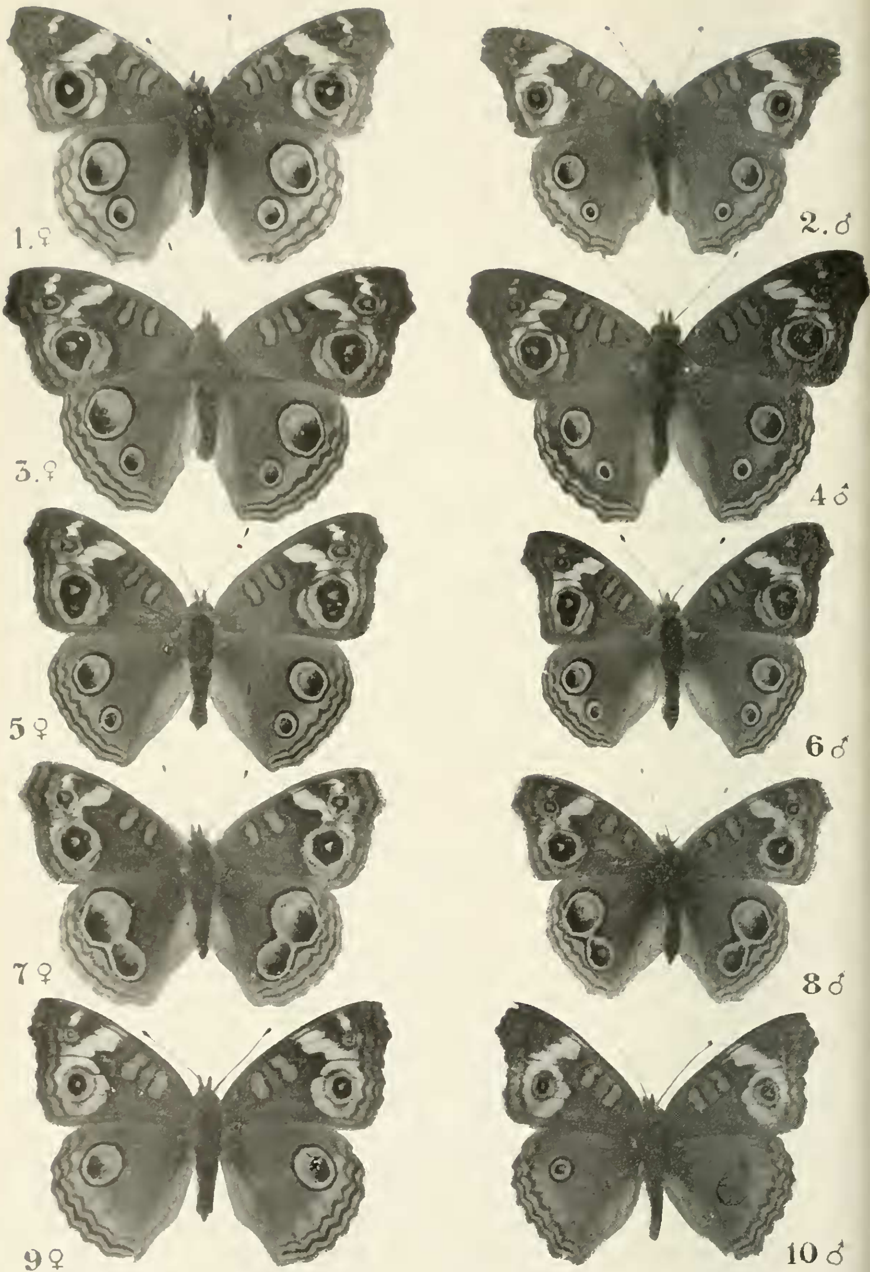

PLATE 1.

sulted from the fact that butterflies live on the average only five days. The two lines would often come to maturity at different times and could not therefore be bred.

The crossing of butterflies of different species has not met with success, largely because of the fact that the genital organs are of quite different types in each species. This does not hold to the same extent for certain groups of large moths, which are more primitive in structure. Prof. Stanfuss, of Zurich, Switzerland, and other experimentors, have met with some success in the latter group. My own work has not included cross-breeding, but has been carried on by intensive inbreeding under varying environments, such as high or low temperatures, increased moisture, or low humidity, exclusion or increase of light, etc.

This has resulted in demonstrating that these environmental combinations have striking results, in combination with selective inbreeding, in changing the colors and markings of butterflies.

Much light has thus been shed on the origin of various changes which make for the production of new species. It can hardly be expected however, even with the application of great patience, that radical changes can be produced in the short span of one life time, when compared with the great amount of time in which nature has operated.

Every collector of Lepidoptera knows that many butterflies produce seasonal forms, the spring generation frequently being quite different from that produced in the summer. There are also many "sports" arising in a state of nature, which frequently vary in a marked degree from the usual insect.

Many of these latter variations arise, so far as my experiments seem to demonstrate, through exposure to heat, from excess of sunlight, at a critical time in the development of the chrysalids. Aberrations of this sort are usually too weak, however, to reproduce themselves.

Junonia coenia has proven the ideal species with which to work. It is longer lived than the average species, the males remaining active about seven days, and the females, when gravid, about two weeks. They become quite tame in captivity, and are easy to handle.

The experiments were begun in a moist air incubator, with a temperature of 80 to 90 deg. Fahr. A complete cycle, from egg to butterfly was carried through in the short span of one month.

This rate could not be maintained however, on account of the development of contagious diseases. The food plant was cultivated in pots to insure its succulency. From 50 to 100 out of each generation were retained, a task that was most difficult on account of the rapid consumption of the plants. When large enough to be easily removed, the young larva were transferred to cut stems of garden grown plants, which had to be renewed daily. The wire cages had to be cleaned every day, to offset the possibility of wilt disease attacking the caterpillars. Various parasites had to be guarded against, and the ants controlled.

Very moist air will, in most butterflies, produce a darker ground color, or an enlargement of dark spots. In Junonia, this change is barely perceptible in the first few generations. A few of the females develop enlarged ocelli on the fore-wings.

These are selected, and paired with males of the same inbred generation. Each successive brood shows an increase in the number of females with enlarged spots on the primaries, and eventually the largest ocellus shows a tendency to develop an appendix. In the course of eight or ten generations a few males will appear with the same changes evident. These, when paired with "sisters" will produce still more startling results, as will be noted on plate 1, No. 3 ♀, a specimen with dark ground color, and black appendices on the ocelli

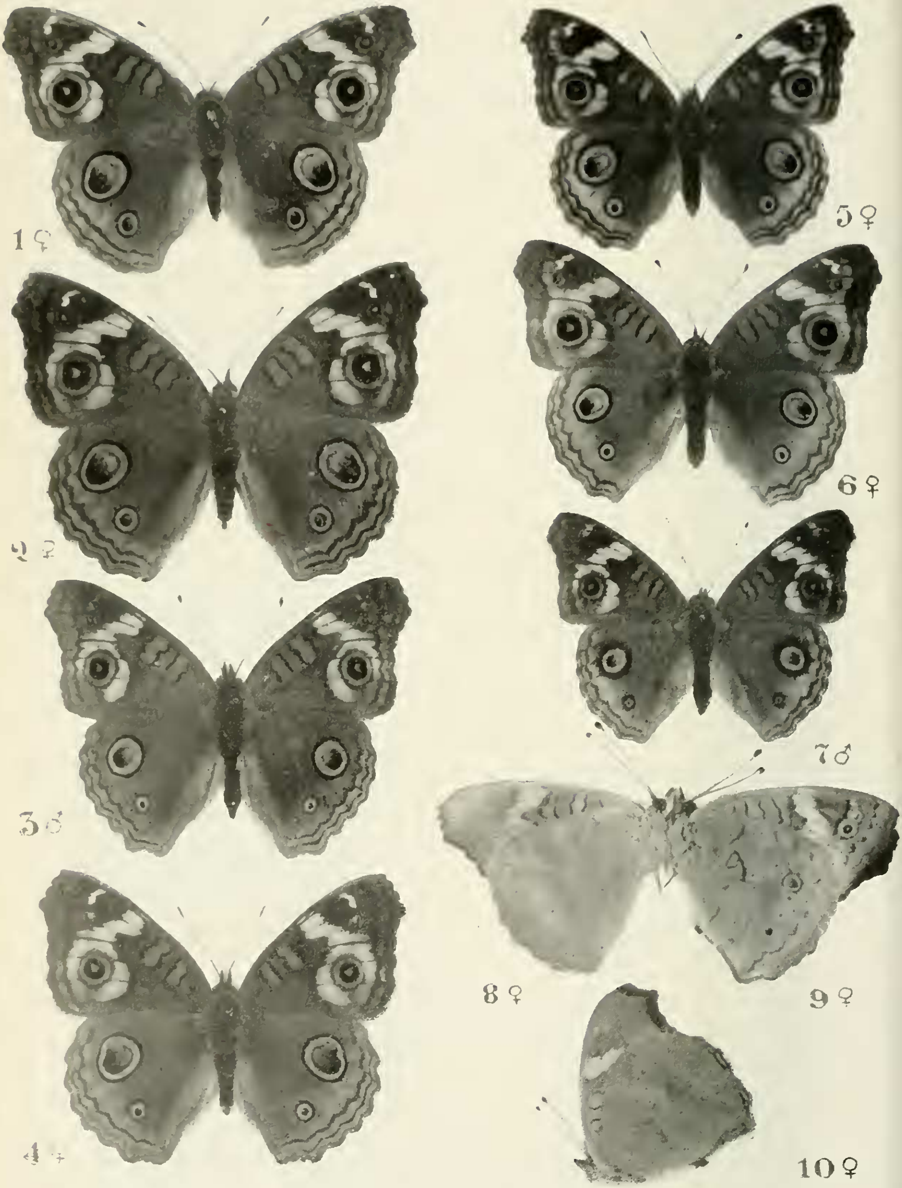

PLATE 2.

of forewings,—and also in specimen No. 4 ♂ of the same plate. Both these specimens are of the tenth generation. It will be noted that these specimens are of unusually large size, contrary to the accepted belief that continued inbreeding causes degeneration. I have succeeded in maintaining the customary size of this species through thirty-five generations.

After the tenth generation occasional females arise in which a light center appears in the aforementioned appendix.

The best of these were selected and paired. In the 25th generation, females were produced with double ocelli, (see plate 1 No. 5 ♀) and males appeared with very large appendices on the ocelli of the forewings. (plate 1, No. 6 ♂.)

In the females a double white spot will be noted near the outer margin in the apex of the forewings. This is the sex mark of the females of Junonia.

Cool Air Experiments

Making a selection of young caterpillars, from the line bred continuously in 80 to 90 deg. I placed these in a specially constructed cool room. The temperature was first regulated to about 70 deg. to stimulate better growth and not make too rapid a change. Following this the temperature was reduced to an average of 50 deg. Each time that the caterpillars were given new food, which was about twice a week, they were placed in 70 deg. temperature for a short time only, to give them a good start in feeding. The chrysalids were maintained uninterruptedly in the cooler temperature. It was found that the life cycle, in these reduced temperatures, was prolonged to about three months, and in cool weather, even longer.

All of the examples which were transferred from warm to cool air as caterpillars, produced striking changes in one generation.

This was evidenced in the enlargement, and frequently, confluence of the ocelli of the secondaries. Note Plate 1, figs. 7 ♀ and 8 ♂. The experiment was frequently repeated, and always with the same results. Protracted continued cool air inbreeding could not be continuously carried on however, as the effect of lower temperatures seemed to destroy the reproductive faculties.

It was also found that chrysalids taken from a line bred continuously in warm air, and transferred to the cool air chamber, would, when hatched, show very little change. This seems to establish the fact that the influences responsible for the change are operative on the caterpillars.

Dry Air Experiments

Plate 1, No. 9 ♀, and No. 10 ♂ are the result of subjection to dry air. It will be noted that all ocelli are greatly reduced, and the smaller ocelli of the hind wings are obsolete.

These results were procured with the use of a 75 watt bulb, in a specially constructed glass incubator. The procedure is difficult, on account of the drying effect on the food plant. This marked change in the size, and obsolescence of ocelli appears even in the first generation, but only from caterpillars descended from butterflies that were raised for many generations in moist air.

I have twice raised six generations in dry air, but the start was made with eggs collected from the outside. In each instance there was only a small amount of reduction in size of the ocelli.

Selective Color Experiments

On July 5th, 1911, in Los Angeles, I captured a fertile female of Junonia, which to all appearances was of the normal form, except that it was of a large size. (See Plate 2, No. 1 ♀.)

From this example a number of eggs were obtained, which were hatched and the caterpillars reared in a temperature of 80 to 90 deg. All of the progeny were of much increased size, as will be noted from Plate 2, Nos. 2 ♀ and 3 ♂. The ground color of these specimens was also darker, and the two orange colored spots of the forewings near the costal margin were increased in size. Another marked feature was the presence of a purplish irridescence near the costal margin of the primaries and around the aforementioned spots.

I was partcularly interested in this irridescence, (which is evanescent, and fades with time) and desired to improve it by selective inbreeding. Experience had demonstrated that a new color development was first evidenced in the females, and only after a considerable amount of selective breeding became apparent in the males.

In the third generation I obtained about 50 per cent of females with this purplish hue. In subsequent generations a few males appeared with the desired color. These were paired with females having the requisite shade. (See plate 2, No. 4 ♀.) In the eleventh generation so much difficulty was experienced with parasites, that the original stock was lost.

On July 12th, 1925, a fertile female was obtained, flying at large. From this example over a hundred eggs resulted, which were separated in two lots. One of these was subjected to moist warm air, the other to dry air with increased temperature. The latter stock was given particular attention, in an effort to increase the yellow coloration.

It was found that the dry warm air chamber markedly accelerated the rate of growth, to such an extent that at the present writing (in a four months period) five generations have reached maturity. This is a record for rapid development.

Plate 2, No. 5 ♀ shows a female of the first generation, raised in the electrically heated incubator, at an average temperature of about 85 deg. A variation of ten degrees one way or another seems to have little effect in producing color changes. This example showed an increase in the yellow coloration over that of the parent, the shade having extended in area.

Plate 2, No. 6 ♀ is of the second generation. In this specimen the yellow extends to the white sex mark in the apex of forewing, the ground color is lighter, and the size increased.

Color Variation

Plate 2, No. 7 ♂ is an example which was selected from the stock in which the yellow coloration was being increased, and was subjected as a chrysalis to direct sunlight with incident increased temperature for two hours daily. This is a difficult procedure, as it usually results in the death of the specimen. The result was a butterfly with darkened ground color, and black borders surrounding the ocelli.

This change probably resulted from the increased temperature applied at a critical time in development.

Plate 2, Nos. 8 ♀, 9 ♀, and 10 ♀ show a remarkable range of variation in the maculation and color of the under surfaces. All are progeny of the female captured July 12, 1925. No. 8 is of the third generation bred in dry air and high temperature. It has adopted a uniform gray color, probably as an adaptation to the increase in light.

No. 9 was bred in moist air, in a dark environment. The ocelli of the upper surfaces are reduced in size.

No. 10, bred in cool air developed the purplish-brown shade on the inferior surface, which is customary with all examples raised under like conditions.